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Effect of Different Bentonite Concentrations on Characteristics of Tilapia (O. Niloticus) Fish Oil

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A B S T R A C T

Tilapia is a freshwater fish that is widely cultivated in Indonesian waters. Tilapia is mostly processed only in the meat, causing a buildup of waste. Waste in tilapia processing is liquid waste and solid waste consisting of head, bones, skin, and offal. Tilapia offal can be reused as fish oil. Fish oil is a fatty acid that contains omega-3. Fish oil that can be consumed must comply with food-grade standards, which go through several stages. One of the stages in the manufacture of fish oil is purification by the bleaching process. The bleaching process using bentonite aims to reduce fatty acids and improve the color of fish oil. This study aimed to determine the effect of the use of bentonite on the characteristics of tilapia fish oil and to determine the best concentration. This study used an experimental laboratory method with a completely randomized design (CRD), using one factor, namely different bentonite concentrations using three replications. The concentrations used were 0%, 3%, 5% and 7%. The test parameters observed were yield, PV test, FFA test, p-Anisidin test, clarity test, TOTOX value, and organoleptic test. Parameter data analysis using ANOVA and using Kruskal-Wallis. The test results on fish oil that the yield value ranges from 65% to 100%. The results of the PV test ranged from 3,30 meq/kg to 7,69 meq/kg , while the FFA value was 1,20% to 2,50. The p-AV test results are 4,47 to 10,95 meq/kg. The result of TOTOX value is 11,07 to 25,75 meq/kg, clarity is 62, 33 to 73,00 %.

Keywords: Tilapia, Fish Oil, Refining, Bentonite

1. Introduction

Nile tilapia is a freshwater fish commodity widely cultivated in Indonesia. It is highly favored by the public due to its affordable market price and substantial nutritional content. This has contributed to an annual increase in Nile tilapia consumption. According to data from the Ministry of Marine Affairs and Fisheries (2018), the production of Nile tilapia has shown consistent annual growth. In 2016, production reached 640,568 tons, increasing to 890,909 tons in 2017. This represents a 39.08% increase in production between 2016 and 2017. The substantial production of Nile tilapia generates significant by-products during processing, primarily due to the exclusive utilization of the fish's flesh. This practice results in the accumulation of waste materials such as heads, fin bones, skin, and viscera. Improper disposal or management of these by-products, particularly viscera, can negatively impact the environment. However, repurposing tilapia viscera presents an opportunity to reduce environmental pollution effectively. As noted by Hildawianti et al. (2018), fish processing waste, especially viscera, remains underexploited. Given their high protein content and abundant unsaturated fats, tilapia viscera hold considerable potential as a resource for producing fish oil.

Crude fish oil obtained through extraction often contains impurities that reduce its quality, necessitating a refinement process. The purification of fish oil aims to enhance its quality to meet the standards set by the International Fish Oil Standards (IFOS). The refinement process involves several stages, including degumming, neutralization, and bleaching. The bleaching process, as conducted in this study, serves to eliminate pigments from the oil, resulting in a clearer product compared to oils refined through previous methods. This improved clarity enhances consumer appeal. Additionally, the bleaching process reduces free fatty acids in the oil, which helps prevent lipid oxidation. The bleaching process involves the addition of adsorbents, which are solid materials capable of absorbing specific components from a fluid phase. Common adsorbents include activated charcoal, bentonite, and zeolite, each of which has distinct advantages and limitations in terms of absorption capacity. According to Polli (2016), bentonite stands out for its advantages, such as a large surface area and the ability to expand easily, making it highly suitable as an adsorbent. In this study, bentonite was selected for use in the purification process due to its superior absorption capacity, which contributes to improved oil quality.

The use of bentonite as an adsorbent in the purification of fish oil has been demonstrated by Hastarini et al. (2012). The addition of adsorbents to fish oil not only improves its color but also reduces other components, such as odor, heavy metals, and oxidation products like peroxide, aldehydes, ketones, and free fatty acids. Additionally, it decreases the phosphatide content in fish oil. The concentration of bentonite used in the purification process is determined through prior studies. For instance, research by Sari et al. (2016) showed that purifying fish oil with 1% bentonite at a heating temperature of 80°C for 30 minutes resulted in a peroxide value of 5.79 meq/kg and a free fatty acid value of 0.76%. Higher concentrations of bentonite further enhance the clarity of fish oil. The aim of this study is to examine the effect of varying bentonite concentrations on the purification of Nile tilapia fish oil. The parameters assessed include yield, free fatty acid content, peroxide value, p-Anisidine value, total oxidation value, clarity, and organoleptic properties of the fish oil.

2. Materials and Methods

Fish Oil Extraction

The fish oil extraction process follows the method outlined by Rozi et al. (2019). The viscera of Nile tilapia are cleaned, with inedible parts such as the intestines removed. The cleaned viscera are then placed in an oven at 90°C for 1 hour. After extraction, the oil is separated from impurities and centrifuged at 3,500 rpm for 10 minutes. The purified oil is stored in dark bottles and kept at low temperatures to maintain its quality.

Fis Oil Purification

The fish oil purification process follows the method described by Sari et al. (2016). The process begins with degumming, which involves adding an 8% NaCl solution and heating at 70°C for 15 minutes, followed by filtration. The next step is neutralization, where a 1N NaOH solution at a 2% concentration is added, heated at 60°C for 1 hour, and mixed with distilled water (aquadest) in a 1:1 ratio. The mixture is then separated using a separating funnel and centrifuged at 3,000 rpm for 10 minutes. The final step is bleaching, which uses activated bentonite at concentrations of 0%, 3%, 5%, and 7%. The mixture is heated and stirred at 60°C for 20 minutes, followed by centrifugation at 3,500 rpm for 10 minutes. The refined oil is stored in dark glass bottles at -18°C until further analysis.

Yield

The yield of oil is determined by comparing the volume of oil extracted to the weight of the raw material, expressed as a percentage. The calculation is performed using the following formula:

Yield (%) =Volume of oil extracted (mL)/Weight of raw material (g) ×100

Peroxide Value

The method of determining the peroxide number uses the principle of titration of iodine released from potassium iodide compounds by peroxide using standard thiosulfate solution as a titrant and starch solution as an indicator. This method detects all substances that oxidize potassium iodide under acidic conditions. A total of 5 g of sample is placed in a 250 ml Erlenmeyer flask, then 30 ml of acetic acid and chloroform solution is added with a ratio of 3: 2, then 0.5 ml of potassium iodide (KI) solution is added, the solution is then shaken carefully to mix, then 30 ml of distilled water is added. Next, titration of the solution with 0.01 N sodium thiosulfate ($Na2S2O3$) is carried out until the solution changes color to yellow, after that 0.5 ml of 1% starch indicator solution is added which will change the color of the solution to blue, titration is then continued while continuing to shake the solution until it changes color to light blue which indicates the release of iodine from the chloroform layer, continue titration carefully until the blue color in the solution disappears. The calculation of the peroxide value is carried out with the following equation:

Peroxide Value (meq/kg) = (S × M × 1000) /Sample Weight

Free Fatty Acid Analysis

Fish oil as much as 14 g was put into a 250 ml Erlenmeyer flask, then 25 ml of 95% ethanol was added and heated at a temperature of 40℃. After that, 2 ml of PP indicator was added and titration was carried out with 0.1 N KOH solution until a pink color appeared and did not disappear for 30 seconds (AOAC, 1995). Determining the FFA number can be calculated using the formula:

FFA Number = (mL KOH × M KOH × 56.1) /Sample Weight (g)

P-Anisidine Value

The p-anisidine value test requires two absorbance measurements using different test solutions. Test solution 1 is prepared by dissolving 1 g of the sample in 25 mL of trimethylpentane. Test solution 2 is prepared by adding 1 mL of a p-anisidine solution (2.5 g/L) to 5 mL of test solution 1, followed by shaking and protecting the mixture from light. A reference solution is prepared by adding 1 mL of the p-anisidine solution (2.5 g/L) to 5 mL of trimethylpentane, followed by shaking and protecting it from light. The absorbance of test solution 1 is measured at 350 nm, while the absorbance of test solution 2 is measured at 350 nm exactly 10 minutes after preparation. The p-anisidine value can be calculated using the following equation:

P-Anisidine Value = (25 ×(1.2 A2 – A1))/G

Total Oxidation Value

The Total Oxidation Value (TOTOX) test is conducted by summing the peroxide value (PV) and the p-anisidine value (p-AV). The calculation formula is as follows:

Total Oxidation Value (TOTOX) = (2PV + p-AV)

Clarity

The clarity test procedure, referring to AOAC (1995). The cuvette is cleaned and filled with the standard solution to be used. The standard is measured until the scale needle indicates 100%. The cuvette containing the standard is then replaced with a cuvette containing the oil sample, and the clarity of the oil is measured in terms of % transmission. The measurement is performed after diluting the oil 10 times by mixing 1 part oil (1 mL) with 9 parts solvent (9 mL). The solvent used is n-hexane. The wavelength applied for measuring the clarity of fish oil is 450 nm.

Data Analysis

Parametric data analysis is used for evaluating results such as yield, peroxide value, and free fatty acid value. If the data shows (P>5%), an Analysis of Variance (ANOVA) test can be conducted. The parametric analysis method applied in this study is ANOVA, which, according to Yatuu et al. (2020), identifies two factors that may contribute to differences in the dependent variable. The Honest Significant Difference (HSD) test is conducted if significant differences are observed. Post Hoc tests are performed to determine differences between treatments.

3. Results and Discussions

The results of the chemical characteristics analysis during the purification of Nile tilapia fish oil using bentonite at different concentrations are presented. The bentonite concentrations applied to the fish oil samples were 0% (K), 3% (A), 5% (B), and 7% (C). The findings show that increasing the bentonite concentration results in lower values for yield, peroxide value (PV), free fatty acid (FFA), p-anisidine value (P-AV), and TOTOX value. The detailed results of the chemical characteristics analysis of the purified fish oil are summarized in Table 1.

Parameter	Fish Oil			
	K	A	B	C
Yield	100.00±0.00^{d}	84.00±2.64^{c}	76.33±3.21^{b}	65.00±3.60^{a}
Peroxide Value	7.69±1.18^{c}	5.97±0.65^{bc}	4.35±0.72^{ab}	3.30±0.12^{a}
Ffa	2.50±0.14^{b}	2.24±0.12^{b}	1.58±0.11^{a}	1.20±0.24^{a}
P-Anisidine	10.90±0.65^{c}	9.20±0.44^{bc}	8.11±0.92^{b}	4.47±0.73^{a}
Totox	25.75±3.49^{c}	21.13±0.49^{bc}	17.39±2.51^{b}	11.07±0.55^{a}
Clarity	62.33±3.78^{a}	67.00±2.64^{ab}	69.33±4.04^{ab}	73.00±2.64^{b}

Description:

- Data are the average results of 3 repetitions ± standard deviation

- Data followed by different lowercase letters indicate significant differences (p<5%)

Yield

The yield testing table demonstrates the influence of bentonite usage on the yield of fish oil samples. The yield of fish oil decreases progressively with the increase in bentonite concentration. This phenomenon occurs because bentonite absorbs components present in the oil. According to Hastarini et al. (2012), during the purification process, the heating stage may lead to weight loss in the oil. Purification is performed to remove impurities from crude oil, which subsequently reduces the oil's weight after the process.

Yield is a critical parameter in fishery product processing, as it indicates the quantity of raw material obtained. The yield of fish oil is influenced by the fat content in the fish's body. Higher fat content in fish results in higher yields of fish oil. According to Andhikawati (2020), the yield of fish oil is affected by several factors, including fat content, water content, and protein content in the fish. The fat content in fish is influenced by the feed provided.

Peroxide Value

The peroxide value (PV) testing table reveals varying results across treatments. Based on normality and homogeneity tests of the peroxide value in purified fish oil samples using bentonite, significant differences were observed among samples K, A, and C. The use of adsorbents during oil purification significantly affects PV, as adsorbents can absorb peroxide components present in the oil. According to Rahayu and Purnavita (2014), the ability of bentonite, activated with acid, to absorb peroxide compounds in oil is attributed to the presence of silanol groups. These silanol groups are formed from the SiO_2 compounds in bentonite, which are activated under acidic conditions.

The decline in fish oil quality is primarily caused by oxidation. Oxidation is influenced by the presence of unsaturated fatty acids and oxygen, where an increase in oxidation results in higher peroxide values. The oxidation process generates hydroperoxides, which contribute to rancidity in oil. According to Suadi et al. (2017), oxidation occurs when oil is exposed to air, leading to the formation of peroxides and hydroperoxides, which ultimately cause rancidity.

Free Fatty Acid Value

The test results table indicates that using different bentonite concentrations affects the free fatty acid (FFA) content in fish oil. The FFA test results show that increasing the bentonite concentration significantly reduces the FFA values in the oil. According to Anwar et al. (2016), the levels of FFA are influenced by the ability of activated bentonite to absorb free fatty acid components. This capability is attributed to the presence of silanol groups Si-OH formed from SiO_2 compounds in bentonite during acid activation. The oxygen atom in the silanol group binds with hydrogen atoms in the carboxyl groups of free fatty acids, allowing adsorption onto the adsorbent surface, thereby reducing the free fatty acid value.

P-Anisidine

The table above demonstrates significant differences in the p-anisidine values among samples K, A, B, and C. The decrease in p-anisidine values is attributed to the increasing concentration of bentonite in the oil, which reduces the presence of hydroperoxide compounds. According to Sembiring et al. (2018), activated bentonite effectively absorbs or reduces oxidation products such as aldehydes, ketones, and alcohols, thereby lowering the p-anisidine values.

The p-anisidine test is conducted to assess secondary oxidation in the oil, which occurs as a result of primary oxidation, leading to the formation of by-products. These by-products, primarily carbonyl compounds, are non-volatile. Primary oxidation produces hydroperoxides, whereas secondary oxidation reactions generate aldehydes in the oil. According to Huli et al. (2014), the p-anisidine test measures the secondary oxidation products formed during the decomposition of hydroperoxides, which result in aldehydes and ketones.

Total Oxidation Value

The table above illustrates varying results, with differences in the total oxidation (TOTOX) values derived from the sum of the peroxide value (PV) and p-anisidine value (p-AV). According to Suseno et al. (2019), the total oxidation value reflects the combined effects of primary and secondary oxidation processes, calculated as 2×PV+p-AV2×PV+p-AV. The TOTOX value is used to measure hydroperoxides and their derivatives, providing insights into the oxidation process.

The TOTOX value results from the sum of the peroxide and p-anisidine test values. An increase in the TOTOX value is caused by high PV and p-AV values. Sample K, which exhibited high PV and p-AV values, consequently showed a high TOTOX value. Conversely, samples with lower PV and p-AV values resulted in lower TOTOX values. As stated by Suseno et al. (2020), the total oxidation value is derived from the combined results of two oxidation parameters, PV and p-AV, in the oil. Fish oil with high PV and p-AV values indicates poor quality, as these parameters signify advanced oxidation and degradation.

Clarity

The table above shows significant differences in clarity values among samples K, A, and C, while no significant difference is observed between samples A and B. The clarity of the oil decreases as the concentration of bentonite increases. This is because the addition of adsorbents like bentonite absorbs impurities in the fish oil and enhances its color. According to Marwati et al. (2015), the improvement in oil clarity is due to bentonite's ability to absorb water present in the oil. Water in the oil contributes to turbidity, and its removal by bentonite results in clearer oil.

The increase in clarity with higher bentonite concentrations is associated with an increase in the bentonite's surface area. The greater surface area enables more impurities to be adsorbed, leading to a significant improvement in the oil's clarity.

4. Conclusions

The conclusion drawn from this study is that the addition of bentonite during the bleaching stage, at concentrations of 0%, 3%, 5%, and 7%, significantly affects the purified Nile tilapia fish oil. Although there were observed a yield decrease with more bentonite concentrations, it is proven that the additional bentonites concentration plays a significant role to decreasing free fatty acid content, peroxide value, p-anisidine value, and total oxidation value. Tilapia fish oil quality could be increased with the application of bentonites as its purifier.

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